GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: August 27, 1979

Project Title: **Laser-Excited Raman Spectroscopy of Biopolymers**

Project No: G-33-G04 Green card

Project Director: **Dr. Nai-Teng Yu**

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences; Bethesda, MD 20014

Agreement Period: From 9/1/79 Until 8/31/80 (09 Year)

Type Agreement: **Grant No. 5 R01 GM18894-09**

Amount: **$76,546 PHS Funds (G-33-G04)**
4,000 GIT Contribution (G-33-346)
$79,546 Total

Reports Required: **Annual Progress Reports with Continuation Applications; Terminal Progress Report upon Grant expiration**

Sponsor Contact Person (s):

Technical Matters

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Bethesda, MD 20014

Program Administrator
Dr. Marvin Cassman
(301) 496-7463

Contractual Matters
(thru OCA)

Evelyn W. Carlin, Grants Mgt. Officer
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National Institute of General Medical Sci.
Bethesda, MD 20014

Grants Management Specialists
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(301) 496-7166

TE: FOLLOW-ON TO PROJECT G-33-G03 (08 YEAR)

Defense Priority Rating: None

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

- Project Director
- Division Chief (EES)
- School/Laboratory Director
- Dean/Director–EES
- Accounting Office
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- Project File (OCA)
- Project Code (GTRI)
- Other

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 7/11/83

Project Title: Laser-Excited Raman Spectroscropy of Biopolymers

Project No: G-33-G04

Project Director: Dr. Nai-Teng-Yu

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences
Bethesda, MD 20014

Effective Termination Date: 8/31/82

Clearance of Accounting Charges: 8/31/82

Grant/Contract Closeout Actions Remaining:

NONE

- ☐ **Final Invoice and Closing Documents**
- ☐ **Final Fiscal Report**
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other _____

NOTE: Follow on project (10 year) - G-33-G05

Assigned to: Chemistry (School/~~Laboratory~~)

COPIES TO:

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Other GTRI

FORM OCA 10:781

Final Report

SECTION IV

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	GM18894-10
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial) Yu, Nai-Teng	PERIOD COVERED BY THIS REPORT: FROM 09/01/79 THROUGH 06/30/80
NAME OF ORGANIZATION Georgia Institute of Technology	

TITLE (Repeat title shown in Item 1 on first page)
Laser-excited Raman Spectroscopy of Biopolymers G-33-604/Yu/Chem.

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1(a). (i) C. C. Askren, Nai-Teng Yu and J. F. R. Kuck, Jr. (1979) "Variation of the Concentration of Sulfhydryl along the Visual Axis of Aging Lenses by Laser Raman Optical Dissection Techniques" Exp. Eye Res. 29, 647.

(ii) Nai-Teng Yu, J. F. R. Kuck, Jr. and C. C. Askren (1979) "Red Fluorescence in Older and Brunescent Human Lenses" Invest. Ophthal. and Visual Sci. 18, 1278.

(iii) A. Lanir and Nai-Teng Yu (1979) "A Raman Spectroscopic Study of the Interaction of Divalent Metal Ions with Adenine Moiety of Adenosine 5'-Triphosphate" J. Biol. Chem. 254, 5882.

1(b). (i) Nai-Teng Yu and R. B. Srivastava (1980) "Resonance Raman Spectroscopy of Heme Proteins with Intensified Vidicon Detectors: Studies of Low Frequency Modes and Excitation Profiles in Cytochrome c and Hemoglobin" J. Raman Spectrosc. (in press).

(ii) Nai-Teng Yu and Motonari Tsubaki (1980) "Resonance Raman Spectra of Manganese Myoglobin and Its Azide Complex. Assignment of a New Charge-Transfer Band to Azide (π) → porphyrin (π^*) Transition" (in press).

(iii) Motonari Tsubaki, Raja B. Srivastava and Nai-Teng Yu (1980) "Temperature Dependence of Resonance Raman Spectra of Azide Metmyoglobin and Methemoglobin. Detection of Resonance-enhanced Bound Azide Vibrations and Iron-Azide Stretch" Biochemistry (submitted).

(iv) R. B. Srivastava, C. Pace and Nai-Teng Yu (1980) "Comparative Raman Studies of Cytochrome b_{562} and cytochrome c" FEBS Letts. (submitted).

2. Mr. C. C. Askren has just completed his M.S. degree in June, 1980. A new graduate student (Ph.D. candidate), Miss Helen C. Mackin, has decided to join my group, working on resonance Raman spectrsocopy of heme proteins and metalloporphyrins.

3. Summary Progress Report:

(1) Objective

a. The overall objectives of the total project are: (a) to develop the techniques and procedures necessary to obtain and interpret the Raman spectra of biopolymers; (b) to derive significant structural information not obtainable by other research techniques; and (c) to correleate the structure-function relationship of biomolecules.

b. Goals set for the current year: (a) to compare low-frequency resonance Raman spectra of cytochrome b_{562} and cytochrome c making possible assignments to Fe-axial ligand modes to cytochrome systems; (b) to initiate resonance Raman studies on cobalt- and manganese-substituted myoglobin and hemoglobin, particularly in the low frequency region; (c) to complete the measurement of excitation profile of the 650 cm^{-1} of bound azide bending vibration in Mn(III)Mb·N_3; and (d) to develop effective cross-linking between T7 DNA and RNA polymerase, using ultraviolet pulsed lasers.

2. Main Scientific Findings and Their Significance:

a. The enhancement of bound azide vibrations at 650 cm^{-1} (dp, bending) and 2039 cm^{-1} (dp, antisymmetric stretch) upon excitation at ~400-460 nm indicates the existence of a new charge-transfer transition in Mn(III)Mb-azide complex. The RR spectra of Mn(III)Mb-azide between 150 and 300 cm^{-1} differ dramatically from those of Fe(III)Mb-azide excited in the 640 nm charge transfer band or near the Soret band. Two lines at 170 and 282 cm^{-1} (both polarized) in the Mn(III)Mb-azide spectra exhibit extremely large resonance enhancements and are unshifted by $^{15}N_3$ isotope substitution. These two lines are assigned to the out-of-plane porphyrin ring vibrations, with the latter involving significant Mn(III)-N (pyrrole) stretch. The enhancement of non-totally symmetric azide modes suggests that the charge-transfer state may be mixed with other excited electronic state (possibly Band Va or VI) via Herzberg-Teller vibronic couplings. The lack of enhancement of the Mn(III)-N_3 stretch leads to our present assignment of azide (π) → porphyrin (π^*) charge-transfer rather than azide (π) → metal (d_{z^2}) or azide (n) → metal (d_{z^2}).

b. The Raman spectrum in the high frequency region (1300-16500 cm^{-1}) of cytochrome b_{562} is quite similar to that of cytochrome c, characteristic of low spin, in-plane iron. However, in the low frequency region (200-800 cm^{-1}) the two spectra are markedly different. The C-S stretching of the peripherial thioether linkages has been assigned. Also, the line-splittings at (348, 358 cm^{-1}), (373,380 cm^{-1}), 392, 400 cm^{-1}) and (414, 423 cm^{-1}) have been observed.

c. We have studied the thermal spin equilibria in azide metmyoglobin and azide methemoglobin. The effect of temperature on Raman intensities permits us to assign lines to either high- or low-spin species. With excitation at 647.1 nm the intensity of an $^{15}N_3$ isotope-sensitive mode at ~411 cm^{-1} was found to increase with decreasing temperature, indicating that its origin may not be the high-spin charge-transfer band at ~640 nm as suggested by Asher and Schuster (1979) (Biochemistry, 18, 5377). Instead, it may be enhanced via the weaker low-spin z-polarized charge-transfer band at ~650 nm (Eaton & Hochstrasser, 1968). Our normal coordinate analysis on the model, azide-Fe-imidazole, and the polarized nature of the line allow us to establish that the ~411 cm^{-1} mode in Fe(III)Mb·N_3 and Fe(III)·Hb·N_3 is assignable to the Fe-N_3 stretch of low-spin species. No internal vibrations of bound azide could be enhanced by excitation at 647.1 nm. However, upon excitation at 406.7 nm we have observed the enhancement of antisymmetric azide stretch (both high- and low-spin), in-plane bending (low-spin) and Fe-N_3 stretch (low-spin), indicating the existence of at least two charge-transfer transitions underlying the strong Soret band. Our resonance Raman data will allow us to distinguish the following types: (1) porphyrin (π) → high-spin Fe(d_π), (2) azide (n) → low-spin iron (d), (3) azide (π) → low-spin iron (d),

and (4) azide (π) → porphyrin (π^*) (high-spin).

3. Research Goals in the Coming Year:

   a. To replace the present SIT (silicon intensified target) detector with a more sensitive "intensified silicon photodiode array detector" (PAR model 1420). This detector covers a wider spectral range 200 nm - 900 nm without scintillator. It has the capability of high speed gating - 5 nsec with high on/off ratio (typical 10^6:1) which allows us to study resonance Raman spectra of many fluorescent biological samples, particularly in the UV region.

   b. To prepare cobalt-substituted oxyhemoglobin for studies of Co-O_2 stretch over a wide wavelength region and to compare the results with Fe-O_2 stretch in oxyhemoglobin.

   c. With the installation of a new detector (PAR model 1420), we will begin to collect data for UV resonance Raman spectra of nucleic acid components (GMP, ATP, etc.).

   d. To study resonance Raman spectra of spinach nitrite reductase which contains a siroheme.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

June 20, 1980
Date

Nai-Teng Yu
Principal Investigator